may be particularly useful in the eradication of malignant tumors for cancer therapy.

2730-Pos Board B700
Direct Effect of Isoflurane on Mitochondrial pH
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Introduction: A decrease in mitochondrial pH (pH_m) inhibits mitochondrial permeability transition pore (mPTP) and has been associated with cardioprotection. The volatile anesthetic isoflurane decreases mitochondrial membrane potential ($\Delta\Psi_m$) and inhibits respiratory complex I. However, the effect of isoflurane on pH_m is unknown. We hypothesized that exposure to isoflurane lowers pH_m in cardiomyocytes and isolated mitochondria. **Methods:** The direct effect of 0.5 mM isoflurane (1 MAC) was tested in cardiomyocytes and mitochondria isolated from adult male Wistar rats. We used fluorescence dyes SNARF-1 and BCECF to measure pH_m in myocytes with a confocal microscope and in mitochondria with a spectrofluorometer, respectively. In myocytes, after baseline recording, cells were superfused with isoflurane for 5 min. In mitochondria, isoflurane was added to mitochondria in the presence of pyruvate/malate (5 mM) and ADP (250 μM ADP). Respiration was recorded using a Clark-type electrode with pyruvate/malat as substrate. **Results:** Exposure of myocytes to isoflurane decreased pH_m 0.09 ± 0.03 pH units ($P<0.05$, n=5). In mitochondria, isoflurane induced decrease in pH_m was 8 ± 2% ($P<0.05$, n=5) of maximal acidification induced by mitochondrial uncoupler FCCP (4 μM). This effect was mimicked when mitochondria were exposed to rotenone (5 μM). Isoflurane inhibited pyruvat/malat-dependent oxygen consumption in the presence and absence of ADP. **Conclusion:** These results suggest that isoflurane decreases pH_m through inhibition of complex I of the electron transport chain. Isoflurane-induced acidification may contribute to the immediate protective effect provided by volatile anesthetics when applied at the onset of cardiac reperfusion after an ischemic event.

2731-Pos Board B701
Heart Ischemia: The Transition from Reversible to Irreversible Myocardial Ischemia is Governed by the Mitochondrial Permeability Transition Pore (mPTP)
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The aim of this study was to evaluate the role of mPTP in the passage from reversible to irreversible injury as function of ischemia duration followed by reperfusion. Rat hearts (n=72) were perfused with the Langendorff technique and subjected to global ischemia during 0 (sham), 10, 20, 30, 40 and 60 min at 37°C, followed by 60min reperfusion. Infarct size was evaluated by triphenyltetrazolium chloride (TTC) staining, and creatinine kinase (CK) and lactate dehydrogenase (LDH) release. Normal heart function and recovery was assessed by Rate-Pressure Product (RPP). Mitochondria function was evaluated by Ca2+ Resistance Capacity (CRC) and mPTP installation. A transition from reversible to irreversible ischemia occurred after a period of 20-30min of ischemia. The functional recovery depends on the duration of the ischemia: 10-20min ischemia RPP (10min 31264 ± 1341, 20min 28578 ± 2176 mmHg/beats/min) had a better functional recovery than longer ischemic periods that displayed dramatic RPP decrease (30min 12183 ± 1895, 40 min 7411 ± 578 and 60min 4916 ± 698 mmHg/min; $p<0.001$ vs. 20 min. ischemia). Ischemia <20 min did not elicit infarct as evaluated by TTC-staining, and CK and LDH-release. In line with this observation, CRC was significantly reduced for ischemia longer than 20 min ($p<0.001$). This study shows a parallel evolution between mitochondrial CRC and the appearance of irreversible damage in the ischemia-reperfused heart. (Values, Mean+SE).

2732-Pos Board B702
Bax C-Terminal Peptide - Insights Into Membrane Interactions
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Multi-cellular organisms eliminate unnecessary or defective cells through a process known as apoptosis. This tightly regulated series of events, leading to the concise shutting down and packaging up of the cellular machinery, is aptly coined ''programmed cell death''. The core apoptotic machinery is composed of members of the Bcl-2 family of proteins. Each protein has a specific yet apparently redundant function, as single pro-apoptotic protein deletions do not render the whole system non-functional. The members act synergistically as initiators, effectors and antagonists of apoptosis. The commitment to self-destruct hinges on Bax, the proverbial ''final straw'', facilitating the abrupt release of mitochondrial matrix proteins, setting off an irreversible avalanche of biochemical events including proteolysis and nuclear fragmentation. Though a wealth of data exists on the apoptotic process in general and the Bcl2 family in particular, the precise mechanism by which Bax interacts with and disrupts the structural integrity of the mitochondrial membrane remains elusive. Structural studies infer that the pro-apoptotic function of Bax is mediated by the insertion of the C-terminal helix as well as helices α5-α6 into the mitochondrial membrane. Conversely, the NMR structure of monomeric Bax clearly shows the hydrophobic α5-α6 helices are completely sequestered within the protein; furthermore, the putative regulatory domain is constrained by the presence of the C-terminal helix tucked solidly into its hydrophobic groove, implicating the need for a major conformational change for those interactions to occur. In order to clarify the mechanism by which Bax interacts with the mitochondrial membrane, we have measured the binding affinity, orientation, and depth of insertion of synthetic peptides, corresponding to the last 25 residues of the Bax C-terminus, to artificial mitochondrial membranes by circular dichroism (CD), resonance energy transfer (RET), fluorescence quenching and attenuated total reflectance-fourier transform infrared spectroscopy (ATR-FTIR).

2733-Pos Board B703
PKA Inhibited The Opening Of Mitochondrial Permeability Tansiton Pore Induced By Cytosolic GSK3β
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It has been reported that cardioprotective intracellular signalings converge on glycogen synthase kinase3β (GSK3β) and that inactivated form of GSK3β (due to the phosphorylation of Ser9 induced by kinases) inhibited the mitochondrial permeability transition pore (mPTP), a key regulator of apoptosis. Here, we hypothesized that PKA could modulate the activities of GSK3β and consequently alters mitochondrial function. To test this, we investigated (1) the opening of the mPTP (measured with fluorescent calcein), and (2) mitochondrial membrane potential (measured with TMRE) in saponin-permeabilized rat cardiomyocyte with a laser scanning confocal microscopy. Our results demonstrated that (1) Active (non-phosporylated) form of GSK3β (10 nM) accelerated calcein leakage from the mitochondria (by 82.4 ± 1.0% of the control, $p<0.01$), and this effect was blocked by CsA (an inhibitor of mPTP: 100 nM) (by 92.3 ± 1.5%, $p<0.01$). (2) SB216763 (an inhibitor of GSK3β: 3 mM) inhibited the opening of mPTP induced by active-GSK3 b (by 93.0 ± 0.9%, $p<0.01$). (3) GSK3β depolarized inner membrane potential (to 63.3 ± 7.3% of the control, $p<0.05$) and this effect was inhibited by CsA (95.9 ± 4.8%, $p<0.01$). (4) PKA catalytic subunit (PKA-cat; 10 U/ml) inhibited both the calcein leakage and membrane potential depolarization induced by active-GSK3β (by 93.6 ± 0.6%, $p<0.01$ and 93.1 ± 1.1%, $p<0.01$, respectively). From these results, we concluded that active form of GSK3β opened mPTP and depolarized inner membrane potential and that these effects were inhibited by the inactivation of GSK3β with PKA.

2734-Pos Board B704
Flash Sniper: Automated Detection and Analysis of Mitochondrial Superoxide Flash
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Mitochondrial superoxide flash is a newly discovered physiological phenomenon reflecting elemental and bursting superoxide production in mitochondria of diverse cells in culture and in vivo (Wang et al, *Cell*, **132**, 279).The discovery of superoxide flash opens a unique window to glean into the mitochondrial ROS signaling and its coupling with energy metabolism,cell fate regulation, and oxidative stress-related diseases. Because of the low frequency and long duration (~20s) of superoxide flash, time-lapse confocal imaging (*xyt*) was employed for data acquisition, resulting in huge data sets. For objective, reproducible and efficient flash identification and measurement, here we develop, validate and implement an automated detection algorithm as well as a software, *Flash Sniper*, which fulfills three basic functions – interactive data priming, flash identification, and flash parameter measurement. The data priming module consists of